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Phylogeny and genetic variation in the spiders of the genus *Ryuthela* (Araneae: Liphistiidae)

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Abstract — The molecular analysis using the partial sequencing data of mt-DNA COI gene and nuclear 28S-rRNA gene is conducted to infer the phylogeney and geographical genetic variations in the genus *Ryuthela*. The genus *Ryuthela* is suggested to be the monophyletic and the most derived group in the subfamily Heptathelinae. *Ryuthela iheyana*, *R. sasakii* and *R. ishigakiensis* are monophyletic groups, respectively, but *R. nishihirai* seems to be paraphyletic to *R. iheyana*. *Ryuthela* spiders appear to be diverged into many phylogroups with strong geographic associations.

Key words — *Ryuthela iheyana*, *Ryuthela ishigakiensis*, *Ryuthela nishihirai*, *Ryuthela sasakii*, COI, 28S, Okinawa

Introduction

Many members of araneomorph spiders are able to disperse great distances by aerial ballooning, but primitive mygalomorph spiders seldom disperse by ballooning (Greenstone et al. 1987). Therefore their interpopulation gene flow may be limited and prone to exhibit population divergence. DNA sequencing data showed that *Aptosticus simus*, the trap door spider with a limited dispersal capability, is geographically subdivided and divergent in the absence of morphological differentiation (Bond et al. 2001). Furthermore, in the trapdoor spider species *Antrodiaetus unicolor*, species-level paraphyly was detected by DNA sequencing data (Hendrixson & Bond 2005).

Although I recognized four species in the genus *Ryuthela* by the male palpal morphology (Tanikawa 2013), species concept based on morphological distinctiveness appears to underestimate true diversity for spiders with limited dispersal capabilities (Bond et. al. 2001). Because the genus *Ryuthela* is trap door spiders with a limited ability to disperse and have only a slight morphological differentiation among species (Tanikawa 2013), as in *Aptosticus* or *Antrodiaetus*, geographical divergence and/or species level paraphyly or polypohyly are expected. I conducted molecular analysis using nuclear 28S-rRNA gene and mitochondrial COI gene to infer the phylogenetic relationships among the species and to assess intraspecific and interspecific genetic variations in the genus *Ryuthela*.

Materials and methods

Sampling. The specimens used in this study were collected from throughout the distribution range of *Ryuthela*, that is, from Okinawajima Is. to Iriomotejima Is. of Ryukyu Isls., the southwest Japan (Fig. 1). Female and juvenile specimens were preserved in 99.5% ethanol at 4°C. Male adult spiders were preserved in 75% ethanol at room temperature, except for dissected right fourth leg preserved in 99.5% ethanol at 4°C. Young males were brought back alive to the laboratory and reared until becoming adults. Two specimens of *Liphistius* sp. from Thailand and Malaysia, three specimens of *Heptathela hangzhouensis* from China, and two specimens of *Heptathela yanbaruensis* from Okinawajima Is. were used as the out group in phylogenetic analysis. Sampling data of the specimens used in this study are shown in Appendix.

DNA extraction, polymerase chain reaction and sequencing. Genomic DNA was extracted from muscle of legs of large individuals or the whole cephalothorax of small individuals using DNeasy Blood & Tissue kit (Qiagen, Inc., Germantown, MD). The mitochondrial cytochrome oxidase subunit I (mt-COI) partial sequences and nuclei 28S-rRNA (28S) partial sequences were used for phylogenetic analysis. Mt-COI was amplified using the primer combination CB1: 5′ — TAT GTA CTA CCA TGA GGA CAA ATA — 3′ (Jermiin & Crozier 1994) with HCOI-2198: 5′ — TAA ACT TCA GGG TGA CCA AAA AAT CA — 3′ (Folmer et al. 1994). The reactants were initially denatured for 2 min at 90°C, proceeded with 40 cycles of 15 sec at 90°C, 20 sec at 50°C, 4 min at 72°C. 28S was amplified using the primer combination ZX1: 5′ — ACC CGC TGA ATT TAA GCA TAT — 3′ with AS8OP1: 5′ — AGA GCC AAT CCT TGT CCC GA — 3′ (Bond & Hedin 2006). The reactants were initially denatured for 2 min at 94°C, proceeded with 40 cycles of 30 sec at 94°C, 20 sec at 50°C, 2 min at 72°C. PCR products were purified using the ExoSAP-IT (GE Healthcare Bio-Sciences, Co.

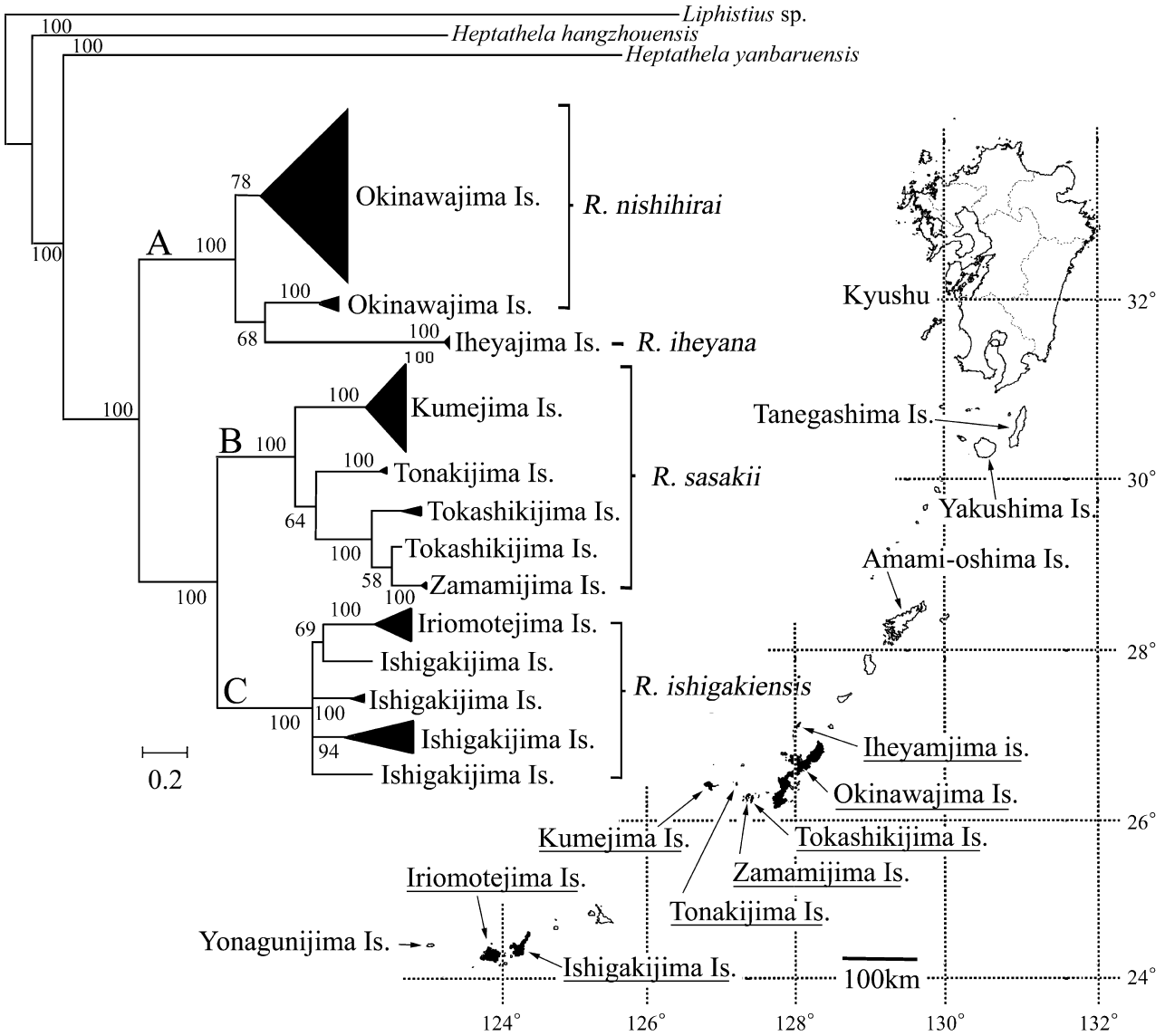

Fig. 1. Left: 50% majority rule consensus tree topologies obtained from Bayesian inference. Posterior probabilities are shown just after nodes. As for the details of topologies and distributions in each island, see figs. 2–4 and appendix. Scale＝0.2 substitution/site. Right: Map showing the Ryukyu Isls. The underlined names show the islands from where *Ryuthela* specimens were collected. Scale＝100 km.

Ltd., Buckinghamshire, England). The purified PCR products were sequenced using the BigDye terminator cycle sequencing kit (ver.3.1) using the primer HCOI-2198 (mt-COI) or ZR1 (28S) and analyzed on ABI 3100 or ABI 3130xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Chromatograms were checked by eye using BioEdit Ver. 7.0.5.0.3 (Hall 1999) or MEGA version 5.05 (Tamura et al. 2011). Sequence alignments were done by MUSCLE (Edgar 2004) in MEGA. Overall mean p-distence was calcurated using MEGA by averaging the number of base differences per site over all sequence pairs.

Phylogenetic analysis. The Perl script KAKUSAN 4 (Tanabe 2011) and TREEFINDER (Jobb et al. 2004) were used to determine the appropriate model of DNA evolution by BIC for Bayesian analyses. MrBays ver. 3.1.2 (Ronquist & Huelsenbeck 2003) was employed to infer the phylogeny on combined data set. Four concurrent Markov Chain Monte Carlo (MCMC) chains were run for 7,000,000 generations, saving a tree every 100 generations. Topologies prior to ln stabilization ("burn-in") were discarded and posterior clade probabilities were computed from the remaining trees. The data of two *Liphistius* specimens were used for root estimation.

Result

In total, I sampled 270 specimens from 75 localities as shown in Appendix and obtained 578 bp of mt-COI, and 697 bp of 28S-rRNA partial sequences from those specimens. About a half (9/20) of 28S sequences showed heterozygote. Overall mean p-distances were 0.122 for mt-

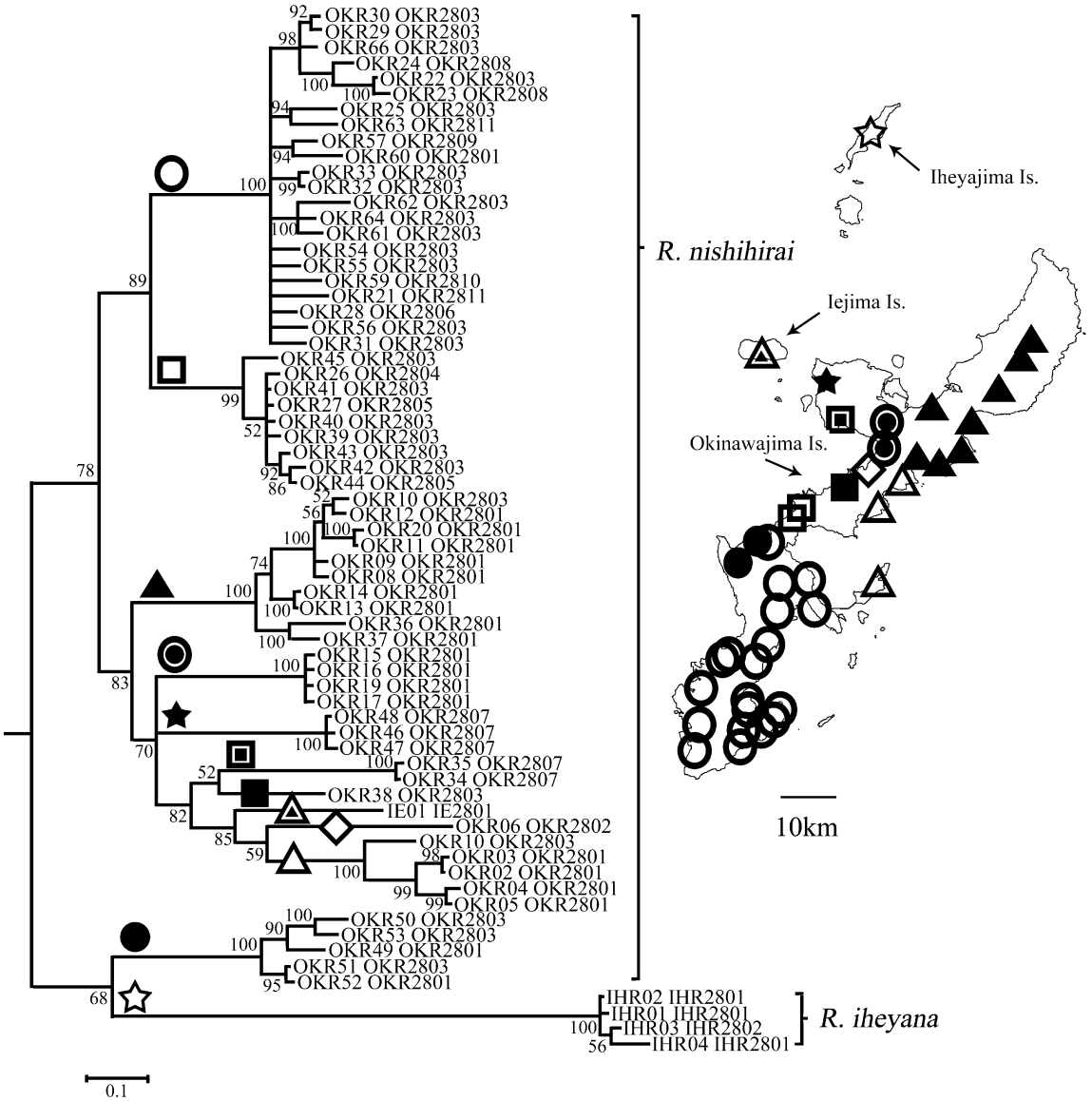

Fig. 2. Left: Details of the topologies of clade A, *R. nishihirai* and *R. iheyana*. The symbols at nodes correspond to their localities shown in right map. The OTUs are shown by the nemes of COI haplotype and 28S genotype. Scale＝0.1 substitution/site. Right: Map showing the localities of specimens belonging to each sub-clade. Scale＝10 km.

COI and 0.0154 for 28S-rRNA. The nucleotide sequence data are available in the DDBJ/EMBL/GenBank databases.

The best-fit models of sequence evolution determined by KAKUSAN 4 were HKY85＋G for 28S, and for mt-COI, SYM＋G for the first codon position, F81＋I for the second position and HKY85＋G for the third position, and gene proportional and codon proportional rather than other patterns of mixed model. Bayesian inference resulted in a phylogenetic tree shown in Figs. 1–4.

Strongly supported (pp＝100) three major clades were recognized in *Ryuthela* (Fig. 1), that is, clade A (comprising *R. nishihirai* and *R. iheyana*), clade B (*R. sasakii*), and clade C (*R. ishigakiensis*). Three species, *R.iheyana*, *R. sasakii* and *R. ishigakiensis*, were inferred to be monophyletic, but *R. nishihirai* seems to be paraphyletic to *R. iheyana*. The geographical population of *R. nishihirai* inferred to be a sister of *R. iheyana* is living in the middle part of Okinawajima Is., far apart from Iheyajima Is. (Fig. 2). *Ryuthela sasakii* was not united with *R. nishihirai* inhabiting nearby but with *R. ishigakiensis* inhabiting in distant islands (Fig. 1). In each major clade, there were many minor clades arising mainly from mt-DNA variability with strong geographic associations (Figs. 2–4), namely, *Ryuthela* populations were geographically divergent, though the relationships among them were ambiguous.

Discussion

The data set of the present study supports the monophyly of the genus *Ryuthela*, while the genus *Heptathela* is not

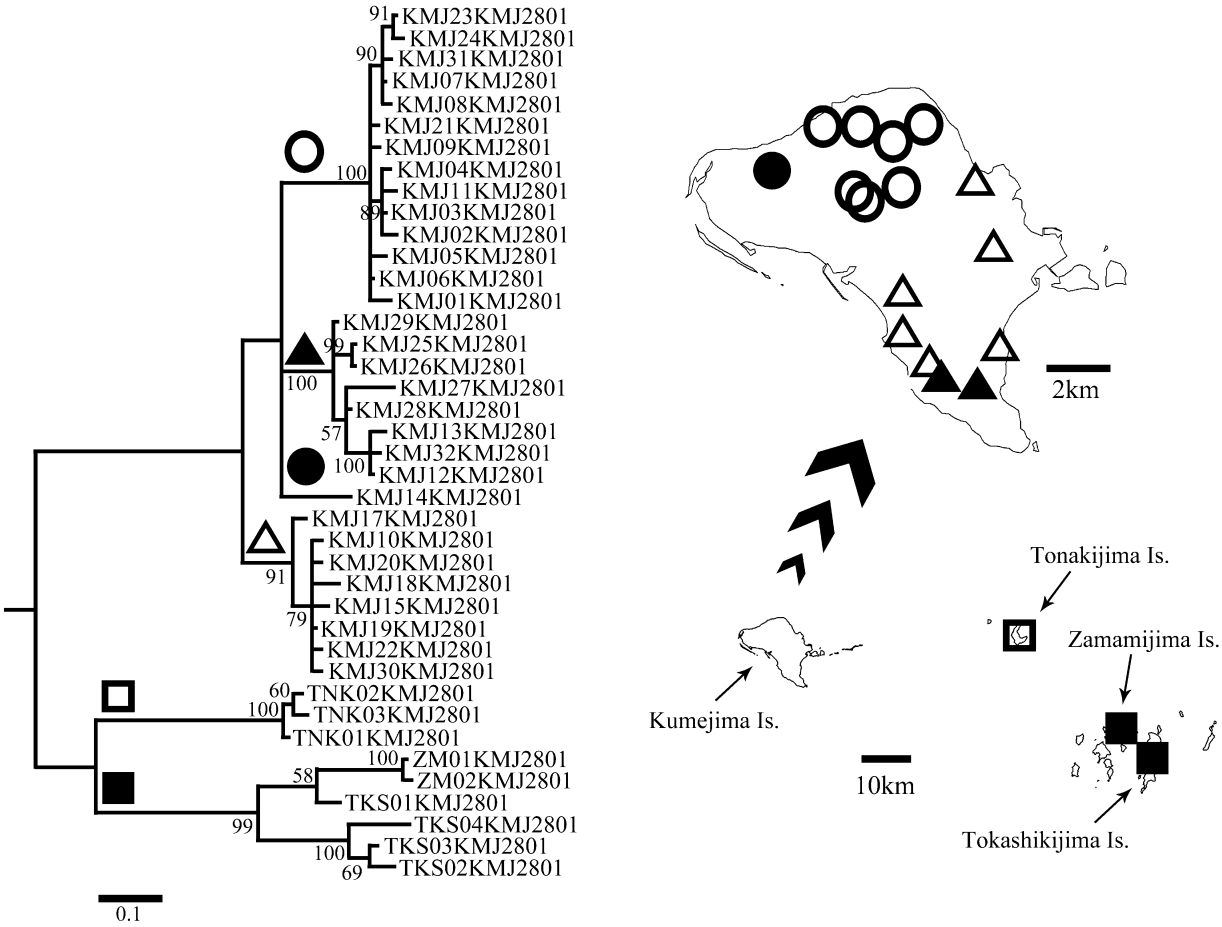

Fig. 3. Left: Detail of the topologies of clade B, *R. sasakii*. The symbols at nodes correspond to their localities shown in right map. The OTUs are shown by the names of COI haplotype and 28S genotype. Scale＝0.1 substitution/site. Right: Map showing the localities of specimens belonging to each sub-clade. Scales＝2 km, 10 km.

monophyletic but is paraphyletic to *Ryuthela*. The phylogenetic position of *Ryuthela* inferred in this study is inconsistent with the cladogram inferred by Haupt using the morphological data (Haupt 2003, fig. 61). The present study shows that *Ryuthela* is a sister group of Japanese *Heptathela* and seems to be the most derived group in the subfamily Heptathelinae, while Haupt's (2003) inference showed that Chinese *Heptathela* (*Sinothela* in Haupt 2003) is a sister of Japanese *Heptathela*, and *Ryuthela* is a sister of all the other members of Heptathelinae (Heptathelidae in Haupt 2003). Haupt mentioned that "*Ryuthela* possibly has hitherto unknown southern connection", but the phylogenetic position inferred in this study is not necessarily in such supposition but connection to the Eastern China along with Japanese *Heptathela*. Further examination should be done in future to clarify the phylogenetic structure of Heptathelinae and the position of *Ryuthela*, including the sequencing data of Vietnamese *Heptathela* (*Nanthela in* Haupt 2003).

There are many minor clades arising mainly from mt-DNA variability, which are distributed geographically nearby (Figs. 2–4), that is, *Ryuthela* spiders, at least females, are diverged into many geographical groups. This mt-DNA divergence supports the low mobility of female spiders as was observed in a previous study (Kikuya 1993). Although such a deep geographical divergence suggests a long history after expanding the distribution of *Ryuthela*, I could not separate them by morphology. There is a possibility of the existence of the cryptic species that are reproductively isolated with no clear morphological differences. However, as the males of *Ryuthela* wander to search for females in the breeding season, nuclear DNA may exhibit a low divergence due to high mobility of males. It is therefore necessary to analyze the population structure using nuclear DNA markers, whose evolutionary rate are much higher than 28S-rRNA gene, to infer the actual amount of gene flow among geographical groups.

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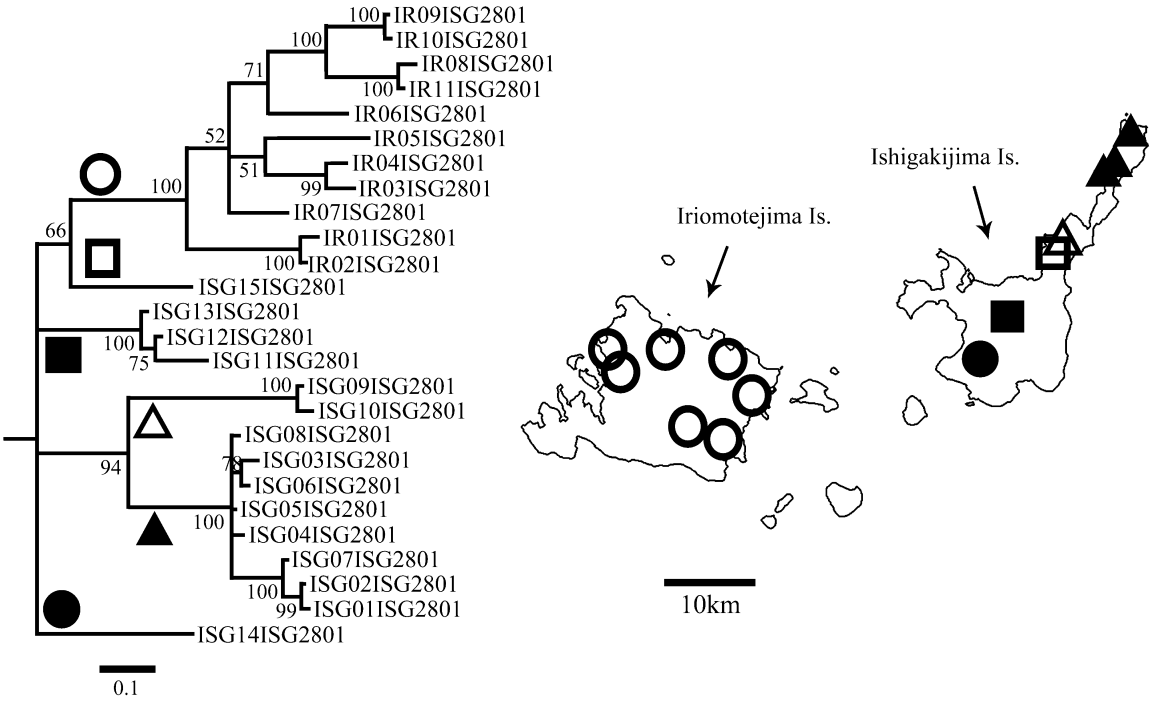

Fig. 4. Left: Detail of the topologies of clade C, *R. ishigakiensis*. The symbols at nodes correspond to their localities shown in right map. The OTUs are shown by the names of COI haplotype and 28S genotype. Scale＝0.1 substitution/site. Right: Map showing the localities of specimens belonging to each sub-clade. Scale＝10 km.

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References

Bond, J. E., Hedin, M. C., Ramirez, M. G. & Opell, B. D. 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. Mol. Ecol., 10: 899–910.

Edgar, C. R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res., 32: 1792–97.

Folmer, O., Black, M., Hoew, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for ampliation of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol., 3: 294–299.

Greenstone, M. H., Morgan, C. E. & Hultsch, A.-L. 1987. Ballooning spiders in Missouri, USA, and New South Wales, Australia: family and mass distributions. J. Arachnol., 15: 163–170.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp., Ser. 41: 95–98.

Haupt, J. 2003. The Mesothelae — a monograph of an exceptional group of spiders (Araneae: Mesothelae): (Morphology, behaviour, ecology, taxonomy, distribution and phylogeny). Zoologica, 154: 1–102.

Hendrixson, B. E. & Bond, J. E. 2005. Testing species boundaries in the *Antrodiaetus unicolor* complex (Araneae: Mygalomorphae: Antrodiaetidae): "Paraphyly" and cryptic diversity. Mol. Phylogenet. Evol., 36: 405–416.

Jermiin L. S. & Crozier, R. H. 1994. The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. J. Mol. Evol., 38: 282–294.

Jobb, G., von Haeseler, A. & Strimmer, K. 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol. Biol., 4: 18

Kikuya, N. 1993. Kimuragumo. Yasaka-shobo, Tokyo, 211 pp. (In Japanese)

Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinforma., 19: 1572–1574.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol., 28: 2731–2739.

Tanabe, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional, and separate models for combined molecular phylogenetic analyses of multilocus sequence data. Mol. Ecol. Resources, 11: 914–921.

Tanikawa, A. 2013. Taxonomic revision of the spider genus *Ryuthela* (Araneae: Liphistiidae). Acta Arachnol., 62: 33–40.

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Appendix. Locality, haplotype, genotype and accession numbers of the specimens used for molecular work.

Locality Latitude Longitude	N	COI Haplotype	Accession No.	28S Genotype	Accession No.
Ryuthela iheyana, Iheyajima Is.					
N27.04599 E127.95997	1	IHR01	AB778118	IHR2801	AB778034
N27.04599 E127.95997	1	IHR01	AB778118	—	
N27.04546 E127.95872	1	IHR01	AB778118	—	
N27.04178 E127.95813	8	IHR02	AB778119	—	
N27.04126 E127.96166	1	IHR02	AB778119	IHR2801	AB778034
N27.04126 E127.96166	4	IHR02	AB778119	—	
N27.04126 E127.96166	1	IHR03	AB778120	—	
N27.03361 E127.95542	1	IHR04	AB778121	IHR2801	AB778034
N27.03361 E127.95542	1	IHR04	AB778121	—	
Ryuthela nishihirai, Okinawajima Is.					
N26.73382 E128.22142	1	OKR12	AB778132	OKR2801	AB778035
N26.72760 E128.21060	1	OKR12	AB778132	—	
N26.68187 E127.89890	1	OKR46	AB778165	OKR2807	AB778041
N26.68187 E127.89890	3	OKR47	AB778166	—	
N26.68187 E127.89890	1	OKR47	AB778166	OKR2807	AB778041
N26.68187 E127.89890	1	OKR48	AB778167	OKR2807	AB778041
N26.67370 E128.18543	1	OKR08	AB778128	OKR2801	AB778035
N26.62611 E127.99059	1	OKR15	AB778135	OKR2801	AB778035
N26.62611 E127.99059	1	OKR16	AB778136	OKR2801	AB778035
N26.62611 E127.99059	1	OKR16	AB778136	—	
N26.62398 E128.06210	1	OKR09	AB778129	OKR2801	AB778035
N26.62398 E128.06210	3	OKR09	AB778129	—	
N26.61970 E127.91851	1	OKR34	AB778153	OKR2807	AB778041
N26.61970 E127.91851	1	OKR35	AB778154	OKR2807	AB778041
N26.60621 E128.14336	1	OKR10	AB778130	OKR2803	AB778037
N26.60621 E128.14336	1	OKR11	AB778131	OKR2801	AB778035
N26.60621 E128.14336	1	OKR20	AB778139	OKR2801	AB778035
N26.58921 E127.99792	1	OKR17	AB778137	OKR2801	AB778035
N26.58921 E127.99792	1	OKR17	AB778137	—	
N26.58921 E127.99792	1	OKR19	AB778138	OKR2801	AB778035
N26.58921 E127.99792	3	OKR19	AB778138	—	
N26.57086 E128.12697	1	OKR13	AB778133	OKR2801	AB778035
N26.57086 E128.12697	1	OKR14	AB778134	OKR2801	AB778035
N26.57086 E128.12697	1	OKR14	AB778134	—	
N26.56284 E127.98829	1	OKR06	AB778127	OKR2802	AB778036
N26.56284 E127.98829	1	OKR06	AB778127	—	
N26.55508 E128.05146	1	OKR36	AB778155	OKR2801	AB778035
N26.55508 E128.05146	1	OKR36	AB778155	—	
N26.54453 E128.08456	1	OKR37	AB778156	OKR2801	AB778035
N26.54453 E128.08456	1	OKR37	AB778156	—	
N26.52048 E128.01381	1	OKR02	AB778123	OKR2801	AB778035
N26.52048 E128.01381	5	OKR02	AB778123	—	
N26.52048 E128.01381	1	OKR03	AB778124	OKR2801	AB778035
N26.50046 E127.91703	1	OKR38	AB778157	OKR2803	AB778037
N26.50046 E127.91703	1	OKR38	AB778157	—	
N26.48550 E127.98256	1	OKR04	AB778125	OKR2801	AB778035
N26.48550 E127.98256	1	OKR05	AB778126	OKR2801	AB778035
N26.48550 E127.98256	3	OKR05	AB778126	—	
N26.46752 E127.83551	1	OKR24	AB778143	OKR2804	AB778038
N26.46752 E127.83551	1	OKR27	AB778146	OKR2805	AB778039
N26.46752 E127.83551	1	OKR39	AB778158	OKR2803	AB778037
N26.46752 E127.83551	2	OKR39	AB778158	—	
N26.46752 E127.83551	1	OKR40	AB778159	OKR2803	AB778037
N26.46752 E127.83551	2	OKR40	AB778159	—	
N26.46752 E127.83551	1	OKR41	AB778160	OKR2803	AB778037
N26.46752 E127.83551	1	OKR42	AB778161	OKR2803	AB778037
N26.46752 E127.83551	1	OKR43	AB778162	OKR2803	AB778037
N26.46752 E127.83551	3	OKR43	AB778162	—	
N26.46752 E127.83551	1	OKR44	AB778163	OKR2805	AB778039
N26.46714 E127.84935	1	OKR45	AB778164	OKR2803	AB778037
N26.42298 E127.78798	1	OKR50	AB778169	OKR2803	AB778037
N26.42298 E127.78798	1	OKR51	AB778170	OKR2803	AB778037

N26.42298 E127.78798	3	OKR51	AB778170	—	
N26.42298 E127.78798	1	OKR52	AB778171	OKR2801	AB778035
N26.42298 E127.78798	1	OKR53	AB778172	OKR2803	AB778037
N26.42298 E127.78798	1	OKR66	AB778183	—	
N26.40668 E127.74247	1	OKR49	AB778168	OKR2801	AB778035
N26.40668 E127.74247	1	OKR49	AB778168	—	
N26.37818 E127.86875	1	OKR54	AB778173	OKR2803	AB778037
N26.37818 E127.86875	1	OKR54	AB778173	—	
N26.36363 E127.81112	1	OKR22	AB778141	OKR2803	AB778037
N26.36363 E127.81112	1	OKR22	AB778141	—	
N26.36363 E127.81112	1	OKR23	AB778142	—	
N26.36363 E127.81112	1	OKR29	AB778148	OKR2803	AB778037
N26.36363 E127.81112	1	OKR29	AB778148	—	
N26.36363 E127.81112	1	OKR30	AB778149	OKR2803	AB778037
N26.36363 E127.81112	1	OKR66	AB778183	OKR2803	AB778037
N26.33230 E127.87889	1	OKR24	AB778143	OKR2808	
N26.33230 E127.87889	1	OKR32	AB778151	OKR2803	AB778037
N26.33230 E127.87889	5	OKR32	AB778151	—	
N26.33230 E127.87889	1	OKR33	AB778152	OKR2803	AB778037
N26.28550 E127.80430	1	OKR25	AB778144	OKR2803	AB778037
N26.28550 E127.80430	1	OKR28	AB778147	OKR2806	AB778040
N26.25936 E127.73562	1	OKR57	AB778176	OKR2809	AB778043
N26.25503 E127.72848	1	OKR60	AB778178	OKR2801	AB778035
N26.25503 E127.72848	1	OKR60	AB778178	—	
N26.25262 E127.77987	1	OKR63	AB778181	OKR2811	AB778045
N26.25262 E127.77987	2	OKR63	AB778181	—	
N26.22829 E127.71476	1	OKR21	AB778140	OKR2811	AB778045
N26.22829 E127.71476	1	OKR21	AB778140	—	
N26.21023 E127.75426	1	OKR55	AB778174	OKR2803	AB778037
N26.21023 E127.75426	2	OKR55	AB778174	—	
N26.18645 E127.76019	1	OKR62	AB778180	OKR2803	AB778037
N26.17239 E127.82384	1	OKR59	AB778177	OKR2801	AB778035
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N26.16368 E127.68427	1	OKR56	AB778175	OKR2803	AB778037
N26.16368 E127.68427	2	OKR56	AB778175	—	
N26.16181 E127.81314	1	OKR31	AB778150	OKR2803	AB778037
N26.16181 E127.81314	1	OKR31	AB778150	—	
N26.15155 E127.76369	1	OKR61	AB778179	OKR2803	AB778037
N26.15155 E127.76369	1	OKR61	AB778179	—	
N26.15155 E127.76369	1	OKR64	AB778182	OKR2803	AB778037
Ryuthela nishihirai, Iejima Is.					
N26.71274 E127.80510	1	IER01	AB778184	IER2801	AB778035
N26.71274 E127.80510	2	IER01	AB778184	—	
Ryuthela nishihirai, Miyagijima Is.					
N26.37323 E127.97456	1	OKR01	AB778122	OKR2803	AB778037
N26.37323 E127.97456	3	OKR01	AB778122	—	
Ryuthela sasakii, Kumejima Is.					
N26.38072 E126.78102	1	KMJ21	AB778204	KMJ2801	AB778047
N26.38038 E126.74975	3	KMJ07	AB778191	—	
N26.38026 E126.76146	1	KMJ23	AB778206	KMJ2801	AB778047
N26.38026 E126.76146	1	KMJ23	AB778206	—	
N26.38026 E126.76146	1	KMJ24	AB778207	KMJ2801	AB778047
N26.37611 E126.77133	1	KMJ05	AB778189	KMJ2801	AB778047
N26.37611 E126.77133	1	KMJ09	AB778193	KMJ2801	AB778047
N26.36829 E126.73401	1	KMJ14	AB778198	KMJ2801	AB778047
N26.36829 E126.73401	4	KMJ14	AB778198	—	
N26.36602 E126.79703	1	KMJ10	AB778194	KMJ2801	AB778047
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N26.36602 E126.79703	1	KMJ20	AB778203	KMJ2801	AB778047
N26.36602 E126.79703	1	KMJ20	AB778203	—	
N26.36346 E126.77384	1	KMJ01	AB778185	KMJ2801	AB778047
N26.36346 E126.77384	1	KMJ06	AB778190	KMJ2801	AB778047
N26.36192 E126.75940	1	KMJ04	AB778188	KMJ2801	AB778047
N26.36192 E126.75940	1	KMJ07	AB778191	KMJ2801	AB778047
N26.36192 E126.75940	1	KMJ07	AB778191	—	
N26.36192 E126.75940	1	KMJ08	AB778192	KMJ2801	AB778047
N26.36192 E126.75940	1	KMJ31	AB778214	KMJ2801	AB778047

N26.36192 E126.75940	3	KMJ31	AB778214	—	
N26.35968 E126.76276	1	KMJ02	AB778186	KMJ2801	AB778047
N26.35968 E126.76276	1	KMJ03	AB778187	KMJ2801	AB778047
N26.35968 E126.76276	1	KMJ03	AB778187	—	
N26.35968 E126.76276	1	KMJ11	AB778195	KMJ2801	AB778047
N26.35968 E126.76276	1	KMJ11	AB778195	—	
N26.34790 E126.80312	1	KMJ17	AB778200	KMJ2801	AB778047
N26.34790 E126.80312	1	KMJ17	AB778200	—	
N26.34790 E126.80312	1	KMJ30	AB778213	KMJ2801	AB778047
N26.33529 E126.77486	1	KMJ15	AB778199	KMJ2801	AB778047
N26.33529 E126.77486	1	KMJ18	AB778201	KMJ2801	AB778047
N26.33529 E126.77486	1	KMJ18	AB778201	—	
N26.32408 E126.77513	1	KMJ19	AB778202	—	
N26.32006 E126.80475	1	KMJ19	AB778202	KMJ2801	AB778047
N26.32006 E126.80475	1	KMJ19	AB778202	—	
N26.31601 E126.78305	1	KMJ22	AB778205	KMJ2801	AB778047
N26.31096 E126.78717	1	KMJ25	AB778208	KMJ2801	AB778047
N26.31096 E126.78717	1	KMJ26	AB778209	KMJ2801	AB778047
N26.31096 E126.78717	1	KMJ27	AB778210	KMJ2801	AB778047
N26.31096 E126.78717	2	KMJ27	AB778210	—	
N26.31096 E126.78717	1	KMJ28	AB778211	KMJ2801	AB778047
N26.30910 E126.79753	1	KMJ12	AB778196	KMJ2801	AB778047
N26.30910 E126.79753	3	KMJ12	AB778196	—	
N26.30910 E126.79753	1	KMJ13	AB778197	KMJ2801	AB778047
N26.30910 E126.79753	1	KMJ29	AB778212	KMJ2801	AB778047
N26.30910 E126.79753	1	KMJ32	AB778215	KMJ2801	AB778047
N26.30910 E126.79753	1	KMJ32	AB778215	—	
Ryuthela sasakii, Tonakijima Is.					
N26.35519 E127.14556	1	TNK01	AB778216	KMJ2801	AB778047
N26.35519 E127.14556	1	TNK02	AB778217	KMJ2801	AB778047
N26.35519 E127.14556	1	TNK02	AB778217	—	
N26.35519 E127.14556	1	TNK03	AB778218	KMJ2801	AB778047
Ryuthela sasakii, Zamamijima Is.					
N26.23177 E127.30678	1	ZM01	AB778219	KMJ2801	AB778047
N26.23177 E127.30678	3	ZM01	AB778219	—	
N26.23177 E127.30678	1	ZM02	AB778220	KMJ2801	AB778047
Ryuthela sasakii, Tokashikijima Is.					
N26.18993 E127.36524	1	TKS02	AB778222	KMJ2801	AB778047
N26.18993 E127.36524	1	TKS02	AB778222	—	
N26.18993 E127.36524	1	TKS03	AB778223	KMJ2801	AB778047
N26.18993 E127.36524	6	TKS03	AB778223	—	
N26.18993 E127.36524	1	TKS04	AB778224	—	
N26.17419 E127.34722	1	TKS04	AB778224	KMJ2801	AB778047
unknown	1	TKS01	AB778221	KMJ2801	AB778047
Ryuthela ishigakiensis, Ishigakijima is.					
N24.59161 E124.31849	1	ISG01	AB778225	ISG2801	AB778048
N24.59161 E124.31849	1	ISG01	AB778225	—	
N24.59161 E124.31849	1	ISG02	AB778226	ISG2801	AB778048
N24.59161 E124.31849	4	ISG02	AB778226	—	
N24.59161 E124.31849	1	ISG07	AB778231	ISG2801	AB778048
N24.56654 E124.30104	1	ISG03	AB778227	ISG2801	AB778048
N24.56654 E124.30104	1	ISG06	AB778230	ISG2801	AB778048
N24.55715 E124.29220	1	ISG04	AB778228	ISG2801	AB778048
N24.55715 E124.29220	1	ISG04	AB778228	—	
N24.55715 E124.29220	1	ISG05	AB778229	ISG2801	AB778048
N24.55715 E124.29220	6	ISG05	AB778229	ISG2801	AB778048
N24.55715 E124.29220	1	ISG08	AB778232	ISG2801	AB778048
N24.49259 E124.24425	1	ISG09	AB778233	ISG2801	AB778048
N24.49259 E124.24425	1	ISG10	AB778234	ISG2801	AB778048
N24.48117 E124.23577	1	ISG15	AB778239	ISG2801	AB778048
N24.48117 E124.23577	1	ISG15	AB778239	—	
N24.41840 E124.18689	1	ISG11	AB778235	ISG2801	AB778048
N24.41840 E124.18689	1	ISG12	AB778236	ISG2801	AB778048
N24.41840 E124.18689	1	ISG13	AB778237	ISG2801	AB778048
N24.37587 E124.15713	1	ISG14	AB778238	ISG2801	AB778048
Ryuthela ishigakiensis, Iriomotejima is.					
N24.38739 E123.75228	1	IR09	AB778248	ISG2801	AB778048

N24.38739 E123.75228	2	IR09	AB778248	—	
N24.38739 E123.75228	1	IR10	AB778249	ISG2801	AB778048
N24.38739 E123.75228	1	IR11	AB778250	ISG2801	AB778048
N24.38516 E123.81487	1	IR01	AB778240	ISG2801	AB778048
N24.38516 E123.81487	1	IR02	AB778241	ISG2801	AB778048
N24.37759 E123.88276	1	IR04	AB778243	ISG2801	AB778048
N24.37759 E123.88276	2	IR04	AB778243	—	
N24.36305 E123.76592	1	IR08	AB778247	ISG2801	AB778048
N24.34300 E123.91141	1	IR03	AB778242	ISG2801	AB778048
N24.34300 E123.91141	1	IR05	AB778244	ISG2801	AB778048
N24.34300 E123.91141	2	IR05	AB778244	—	
N24.31222 E123.84026	1	IR07	AB778246	ISG2801	AB778048
N24.31222 E123.84026	2	IR07	AB778246	—	
N24.29813 E123.87680	1	IR06	AB778245	ISG2801	AB778048
N24.29813 E123.87680	1	IR06	AB778245	—	
Out group					
Heptathela yanbaruensis, Okinawajima Is.					
N26.61563 E128.09049	1	OKHY112	AB778251	OKHY2801	AB778049
N26.83633 E128.27544	1	OKHY203	AB778252	OKHY2801	AB778049
Heptathela hangzhouensis, Changsha, China					
unknown	1	CH01	AB778253	CH2801	AB778050
unknown	1	CH02	AB778254	CH2802	AB778051
unknown	1	CH03	AB778255	CH2801	AB778050
Liphistius sp., Thailand					
unknown	1	LT02	AB778256	LT2801	AB778052
Liphistius sp., Malaysia					
unknown	1	LM01	AB778257	LM2801	AB778053